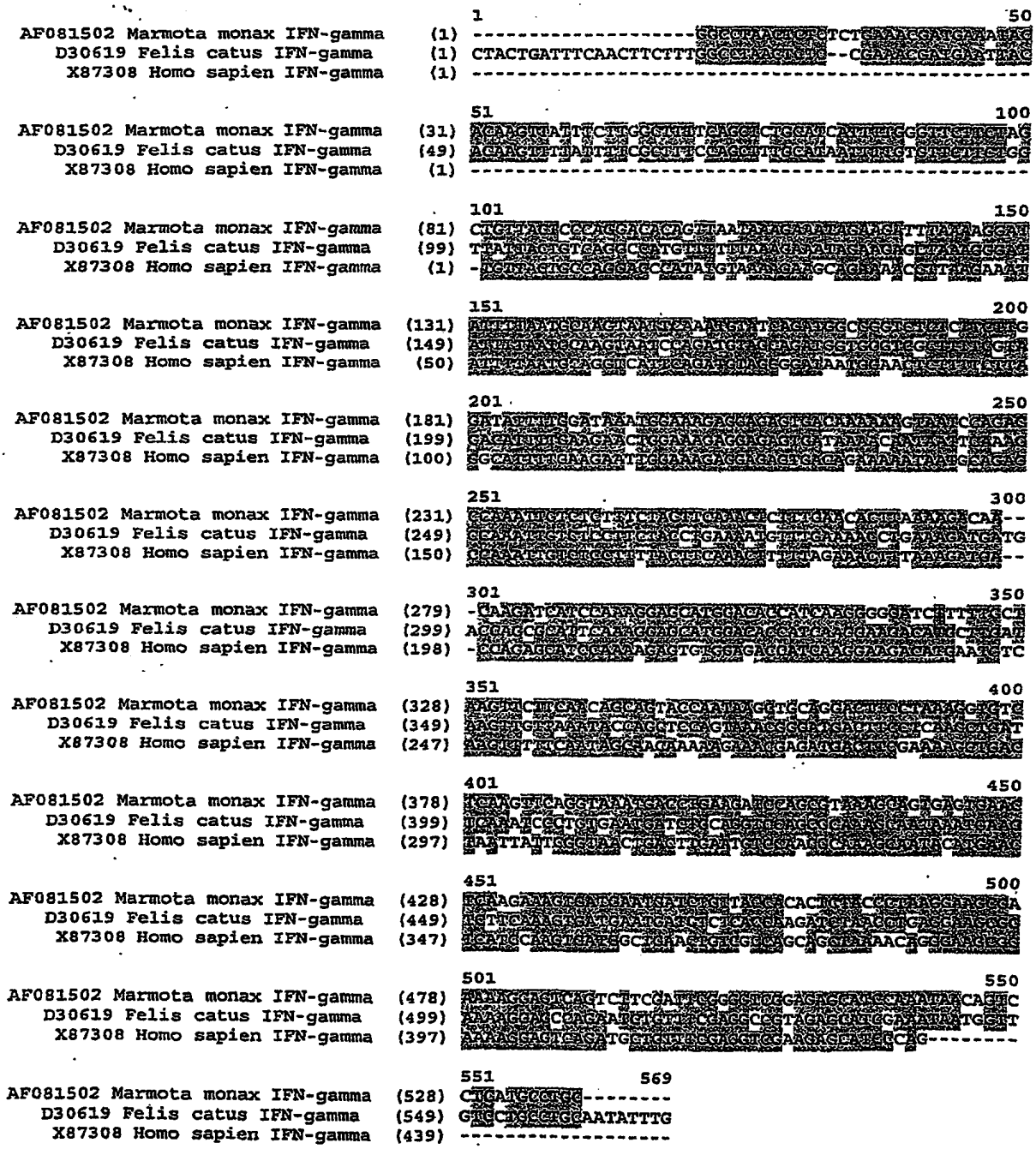
Figure 42

Figure 42: An alignment of the IFN-gamma nucleotide sequences from human, cat, rodent species.



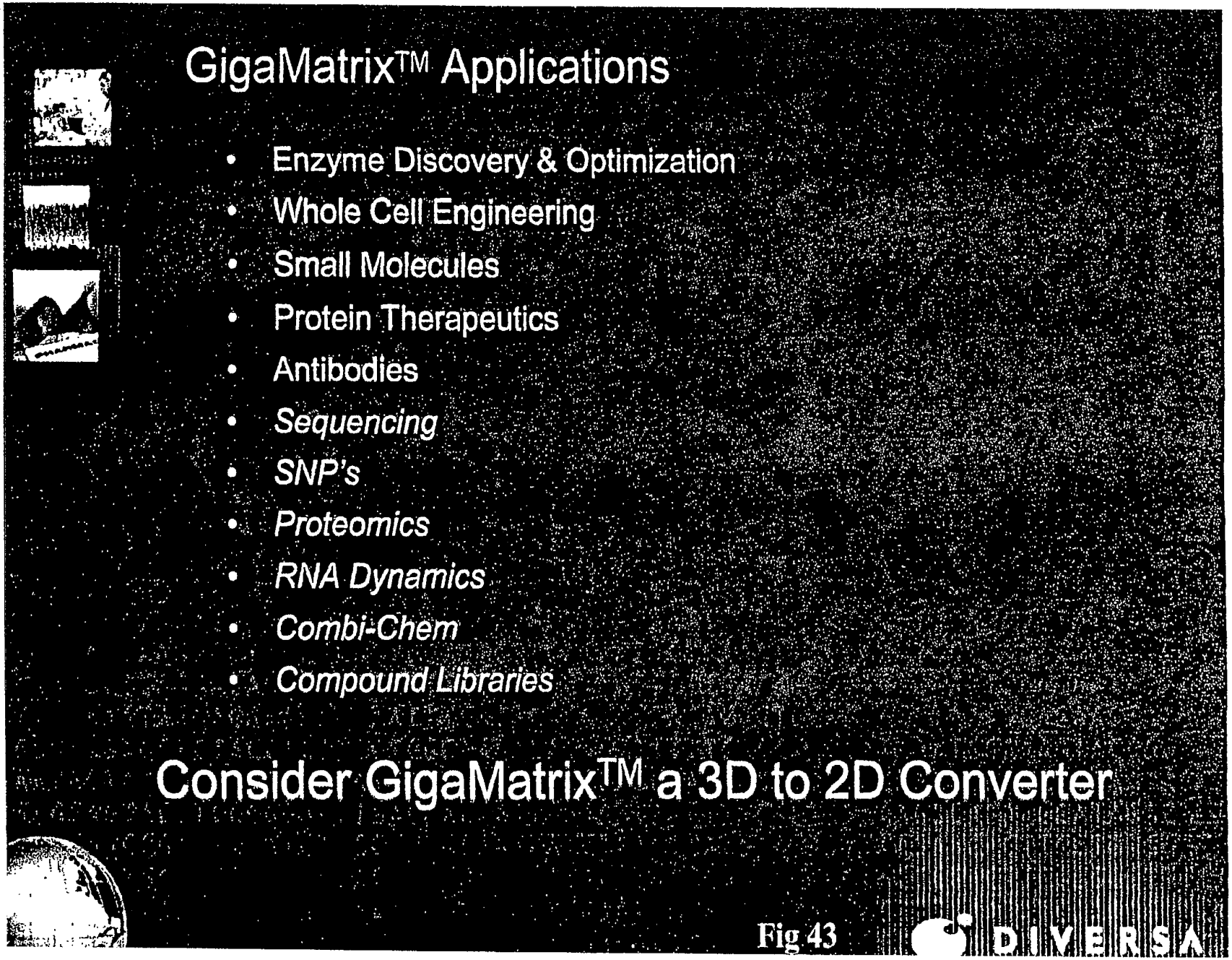

GigaMatrix™ Applications
• Enzyme Discovery & Optimization
• Whole Cell Engineering
• Small Molecules
• Protein Therapeutics
• Antibodies
• Sequencing
• SNP's
• Proteomics
• RNA Dynamics
• Combi-Chem
• Compound Libraries
Consider GigaMatrix™ a 3D to 2D Converter
DIVERSA

Fig 43

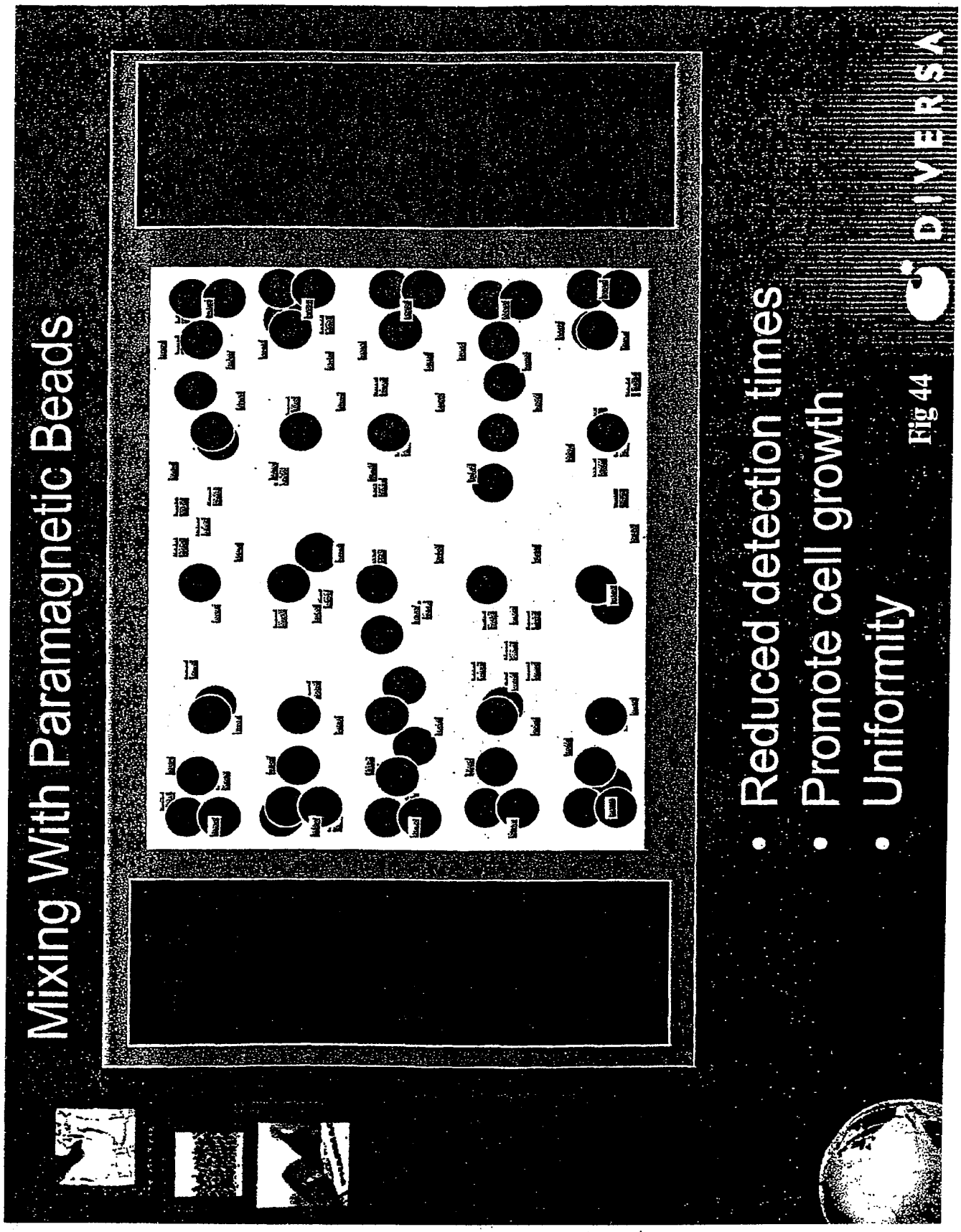

Mixing With Paramagnetic Beads
• Reduced detection times
• Promote cell growth
• Uniformity
DIVERSA

Fig 44

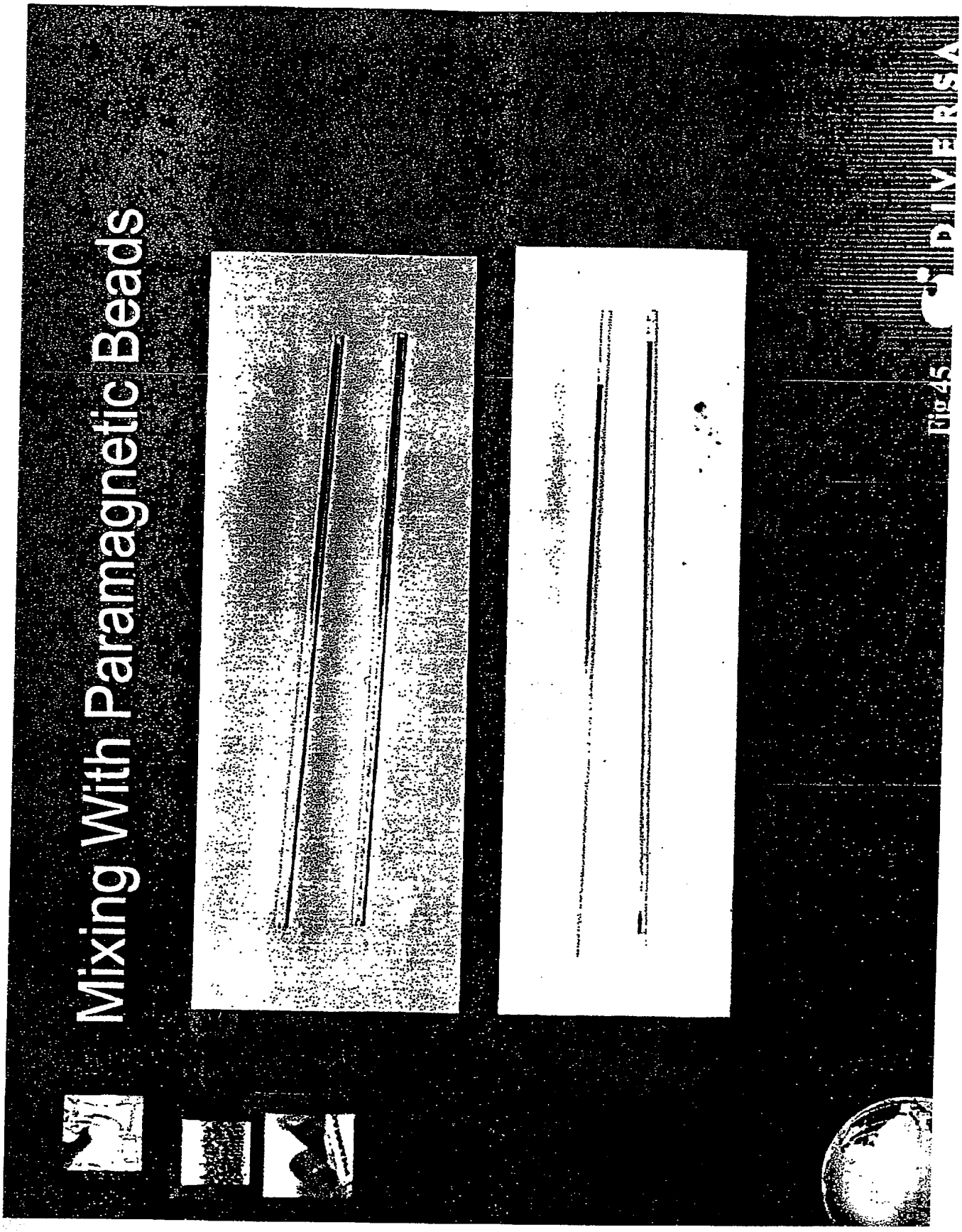


Fig 45

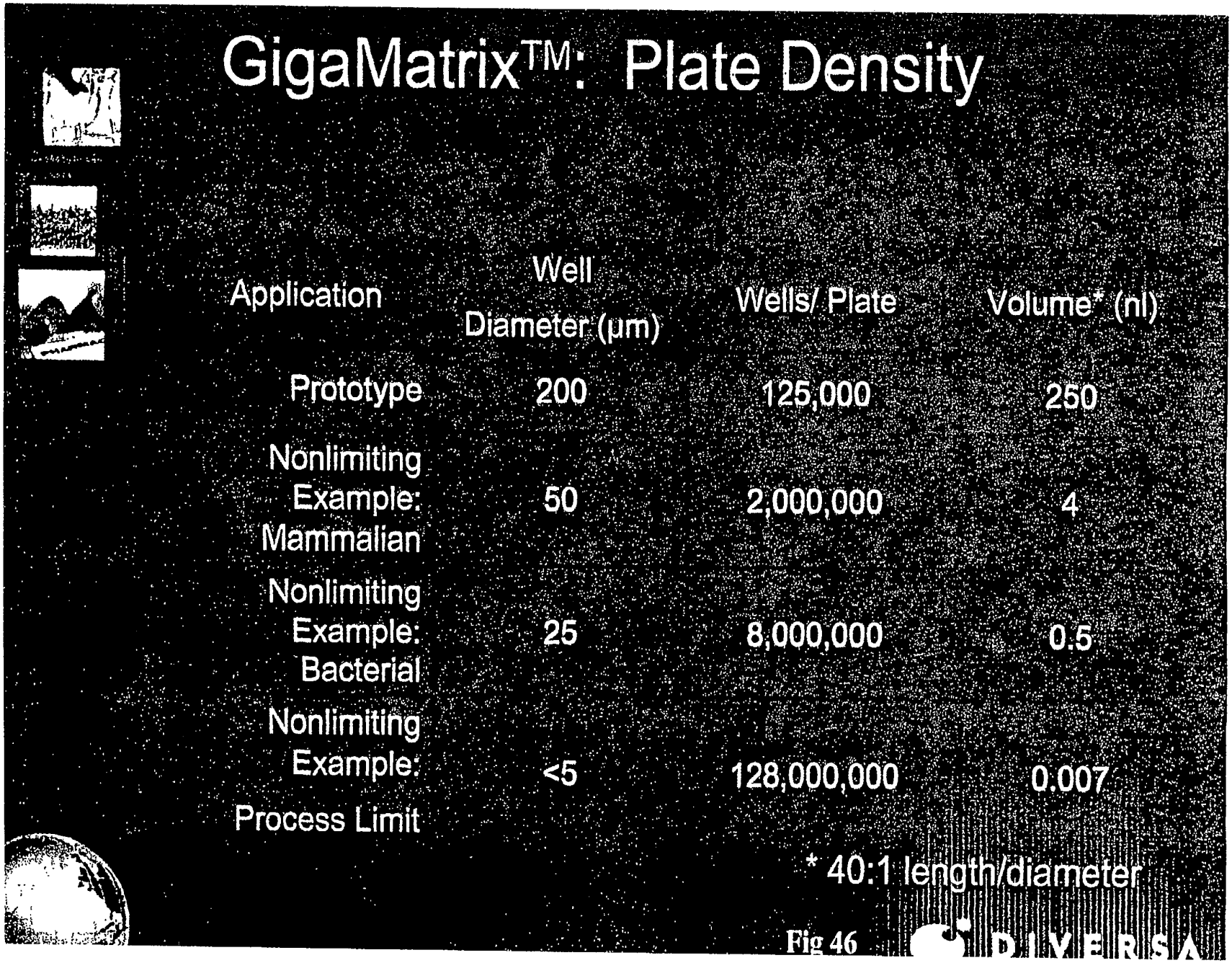

GigaMatrix™: Plate Density
Application
Well Diameter (µm)
Wells/ Plate
Volume* (nl)
Prototype
200
125,000
250
Nonlimiting Example: Mammalian
50
2,000,000
4
Nonlimiting Example: Bacterial
25
8,000,000
0.5
Nonlimiting Example: Process Limit
<5
128,000,000
0.007
* 40:1 length/diameter
DIVERSA

Fig 46

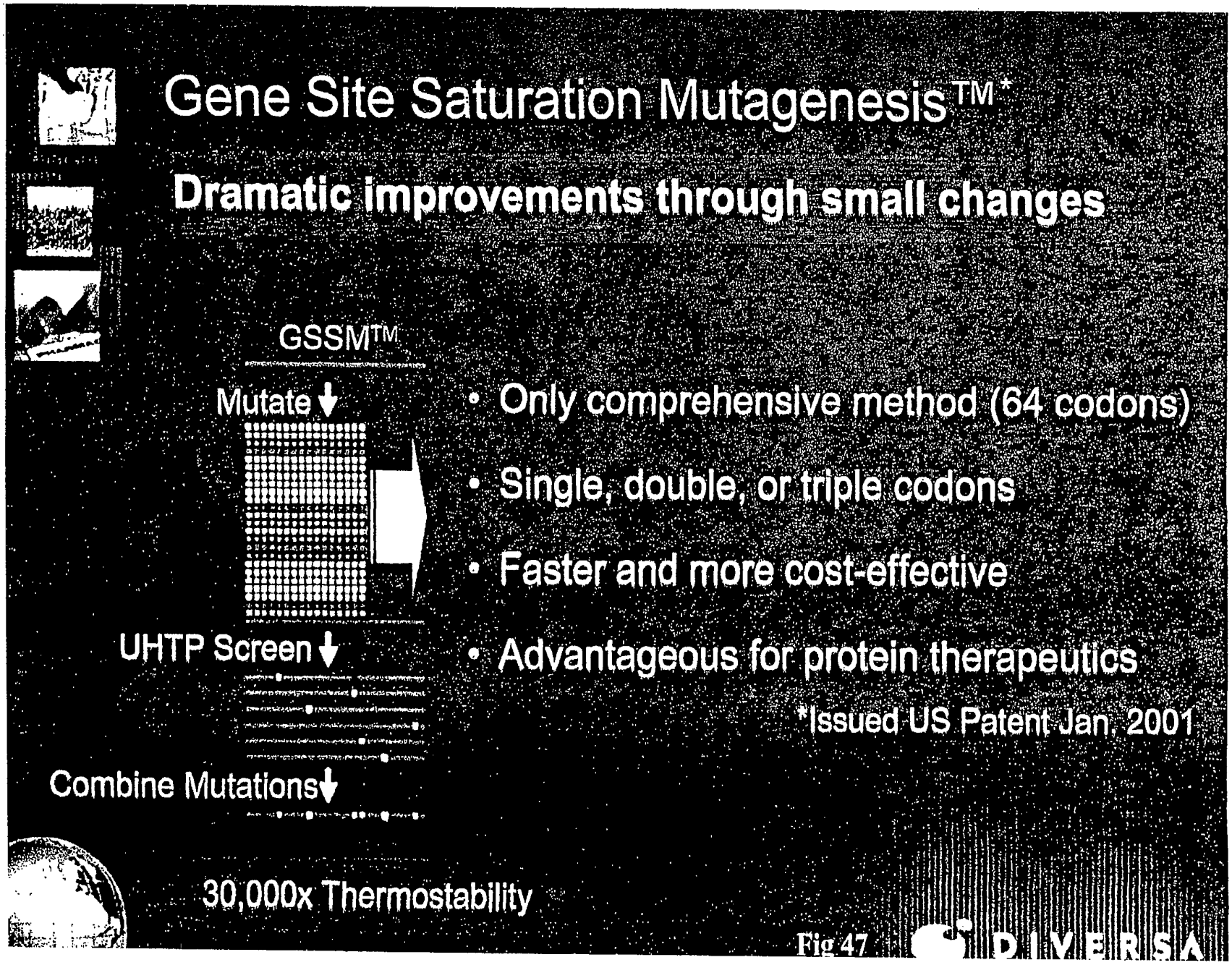

Gene Site Saturation Mutagenesis™*
Dramatic improvements through small changes
GSSM™
Mutate
UHTP Screen
Combine Mutations
30,000x Thermostability
• Only comprehensive method (64 codons)
• Single, double, or triple codons
• Faster and more cost-effective
• Advantageous for protein therapeutics
*Issued US Patent Jan. 2001
DIVERSA

Fig 47

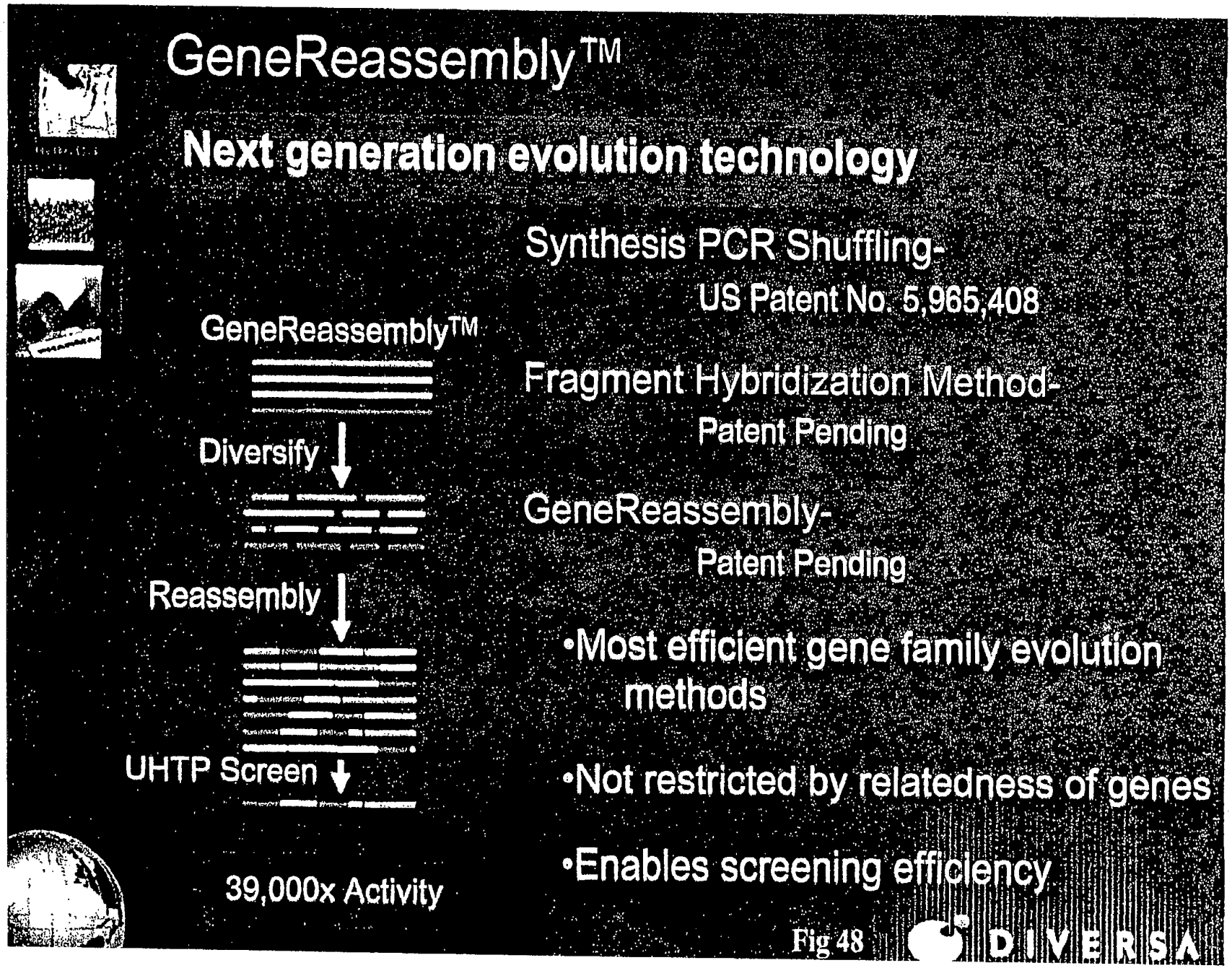

GeneReassembly™
Next generation evolution technology
GeneReassembly™
Diversify
Reassembly
UHTP Screen
39,000x Activity
Synthesis PCR Shuffling-
US Patent No. 5,965,408
Fragment Hybridization Method-
Patent Pending
GeneReassembly-
Patent Pending
•Most efficient gene family evolution methods
•Not restricted by relatedness of genes
•Enables screening efficiency
DIVERSA

Fig 48

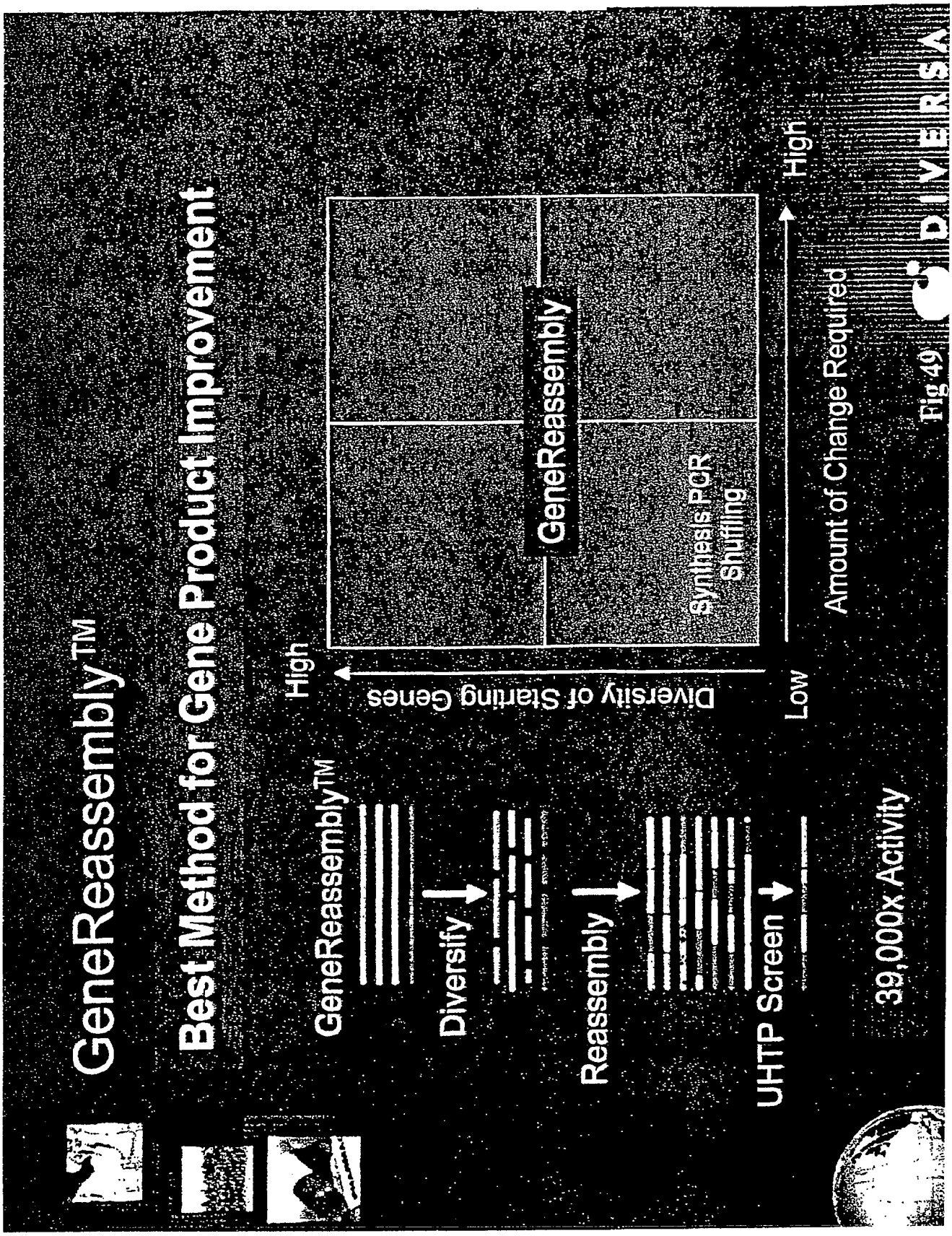

GeneReassembly™
Best Method for Gene Product Improvement
GeneReassembly™
Diversify
Reassembly
UHTP Screen
39,000x Activity
High
Diversity of Starting Genes
Low
GeneReassembly
Synthesis PCR
Shuffling
Amount of Change Required
High
DIVERSA

Fig 49

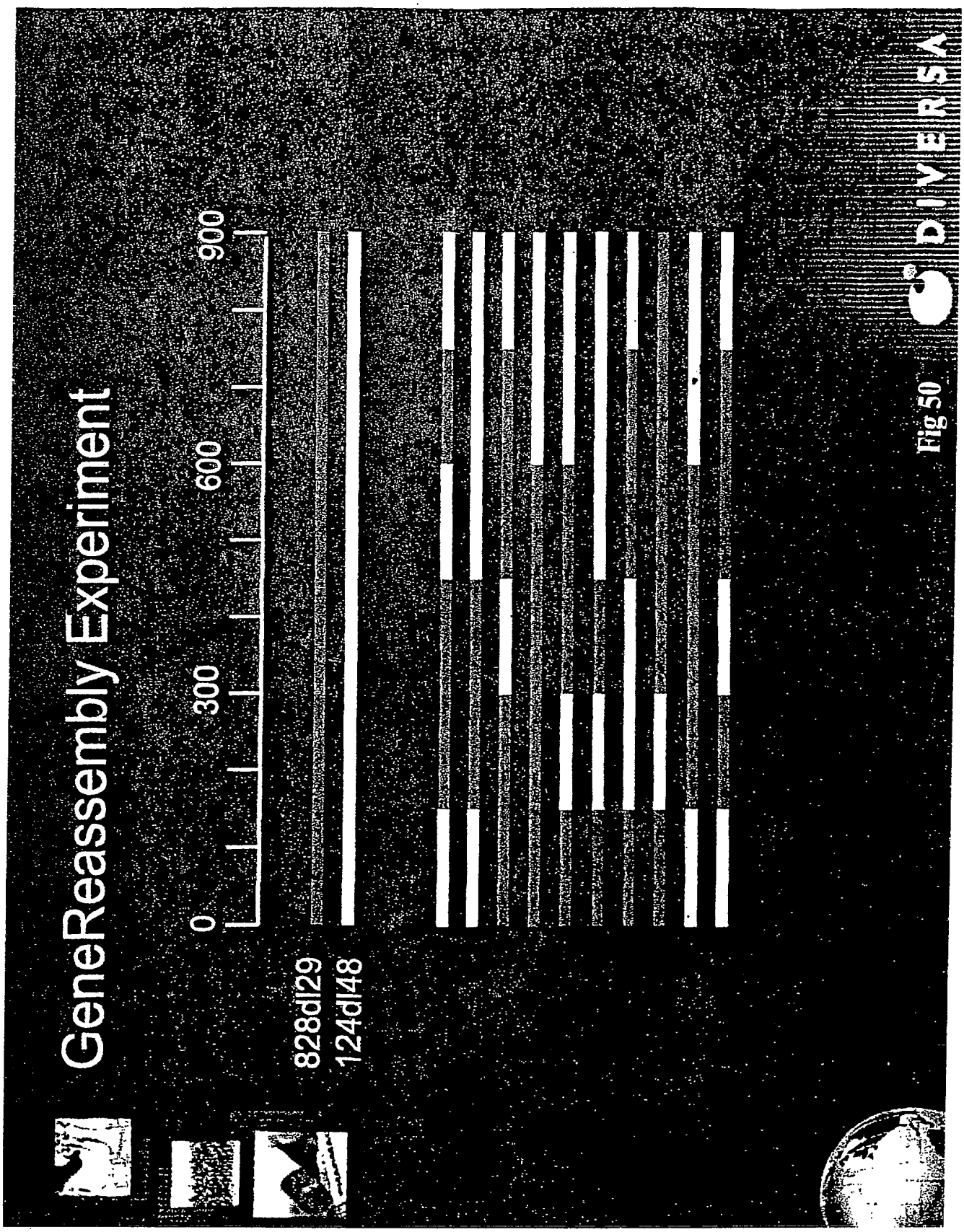

GeneReassembly Experiment
0
300
600
900
828dl29
124dl48
DIVERSA

Fig 50

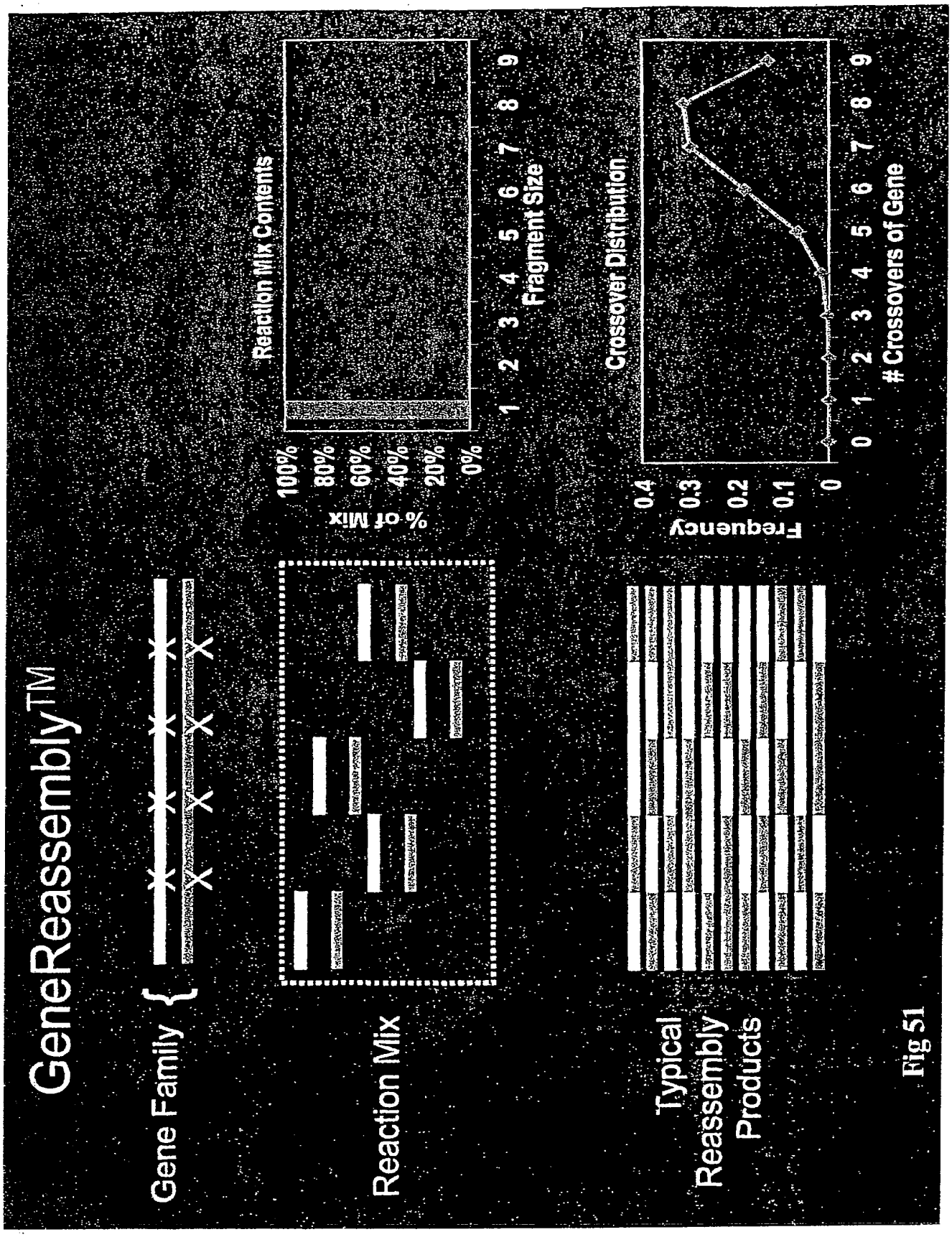

GeneReassembly™
Gene Family
Reaction Mix
Typical Reassembly Products
Reaction Mix Contents
% of Mix
100%
80%
60%
40%
20%
0%
1 2 3 4 5 6 7 8 9
Fragment Size
Crossover Distribution
Frequency
0.4
0.3
0.2
0.1
0
0 1 2 3 4 5 6 7 8 9
Crossovers of Gene

Fig 51

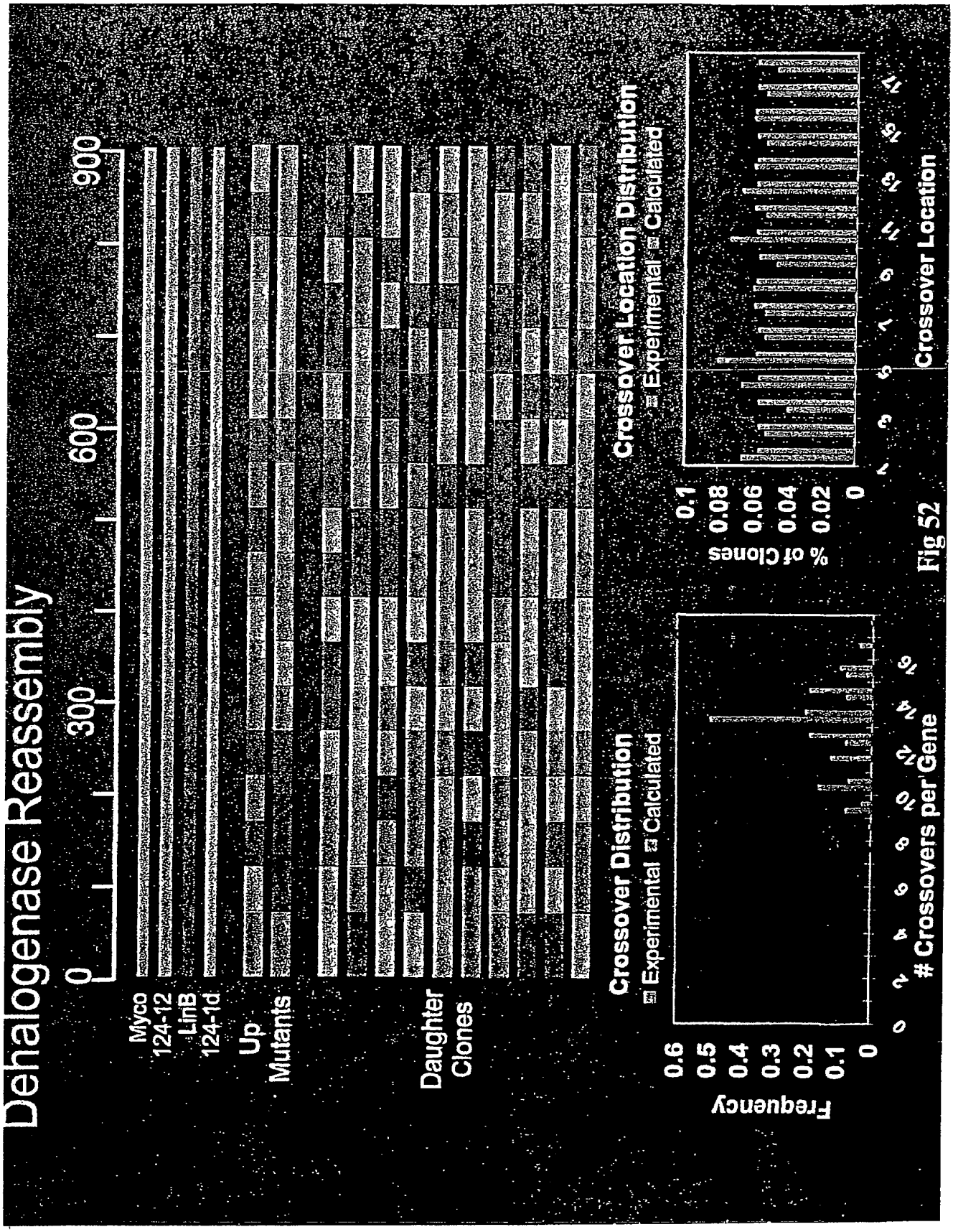

Dehalogenase Reassembly
0
300
600
900
Myco 124-12
LinB 124-1d
Up Mutants
Daughter Clones
Crossover Distribution
Experimental
Calculated
Frequency
Crossovers per Gene
Crossover Location Distribution
Experimental
Calculated
% of Clones
Crossover Location

Fig 52

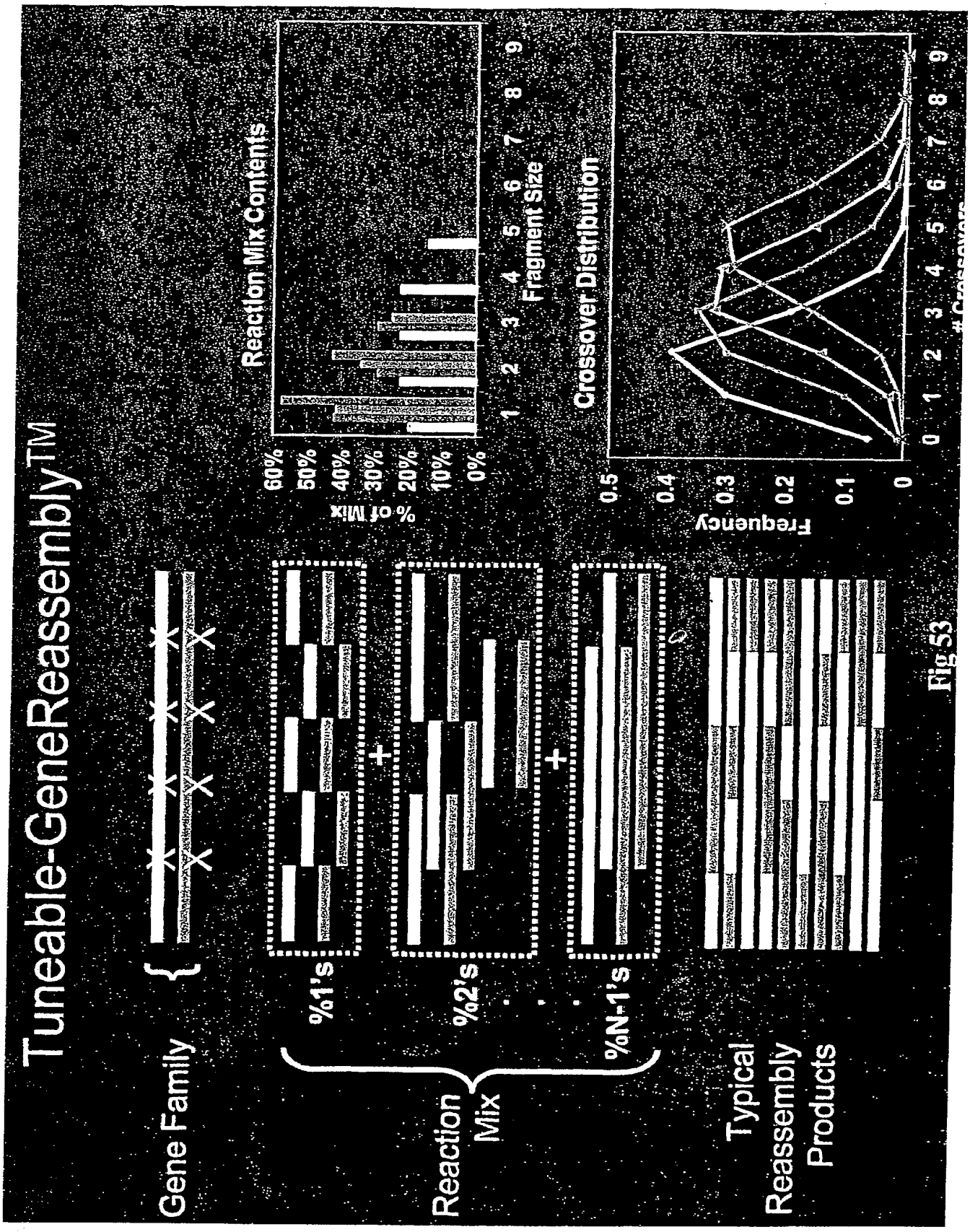

Tuneable-GeneReassembly™
Gene Family
Reaction Mix
%1's
%2's
%N-1's
Typical Reassembly Products
Reaction Mix Contents
% of Mix
60%
50%
40%
30%
20%
10%
0%
1 2 3 4 5 6 7 8 9
Fragment Size
Crossover Distribution
Frequency
0.5
0.4
0.3
0.2
0.1
0
0 1 2 3 4 5 6 7 8 9

Fig 53

DNACarpenter™ – Reassembly Control Software

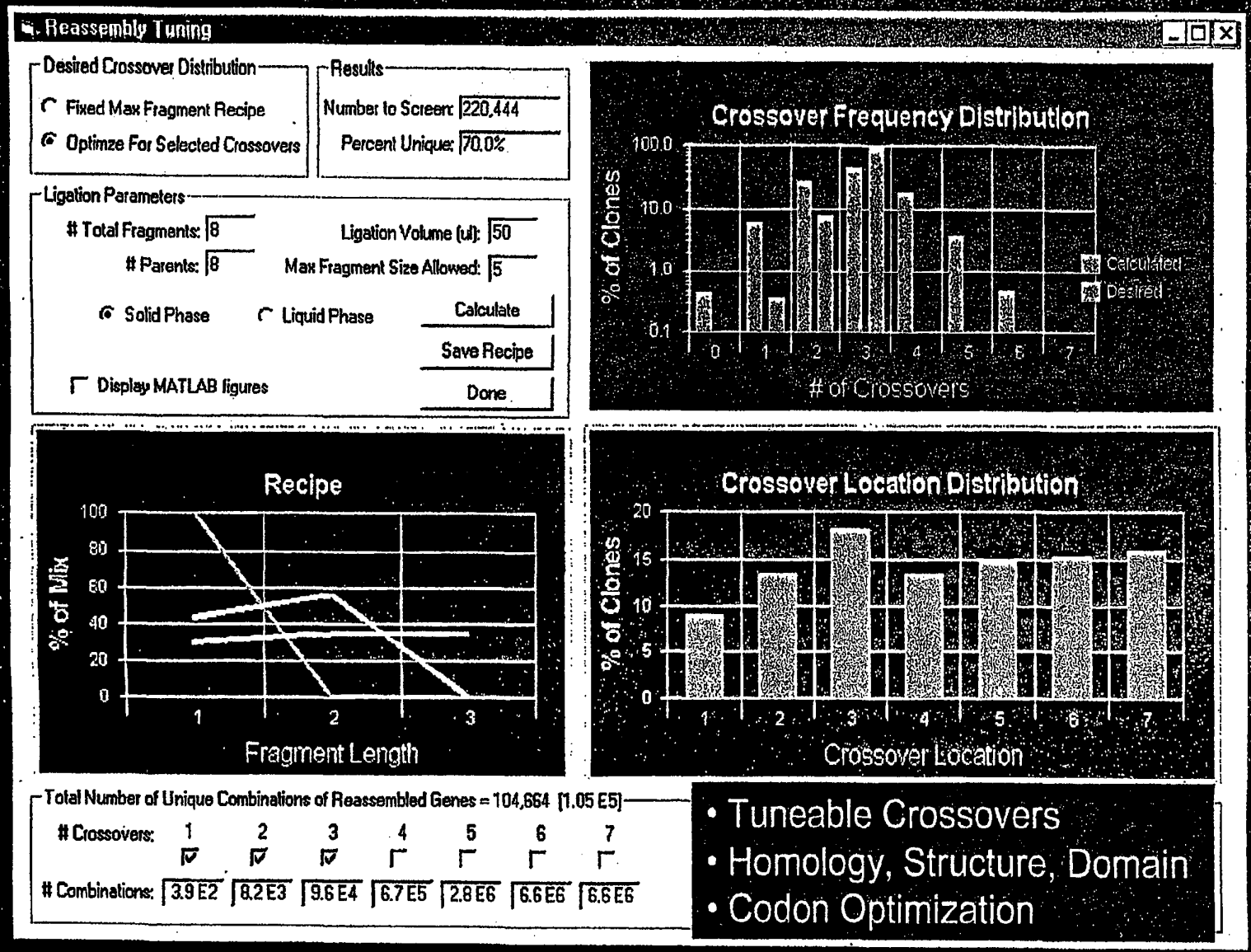


Fig 54

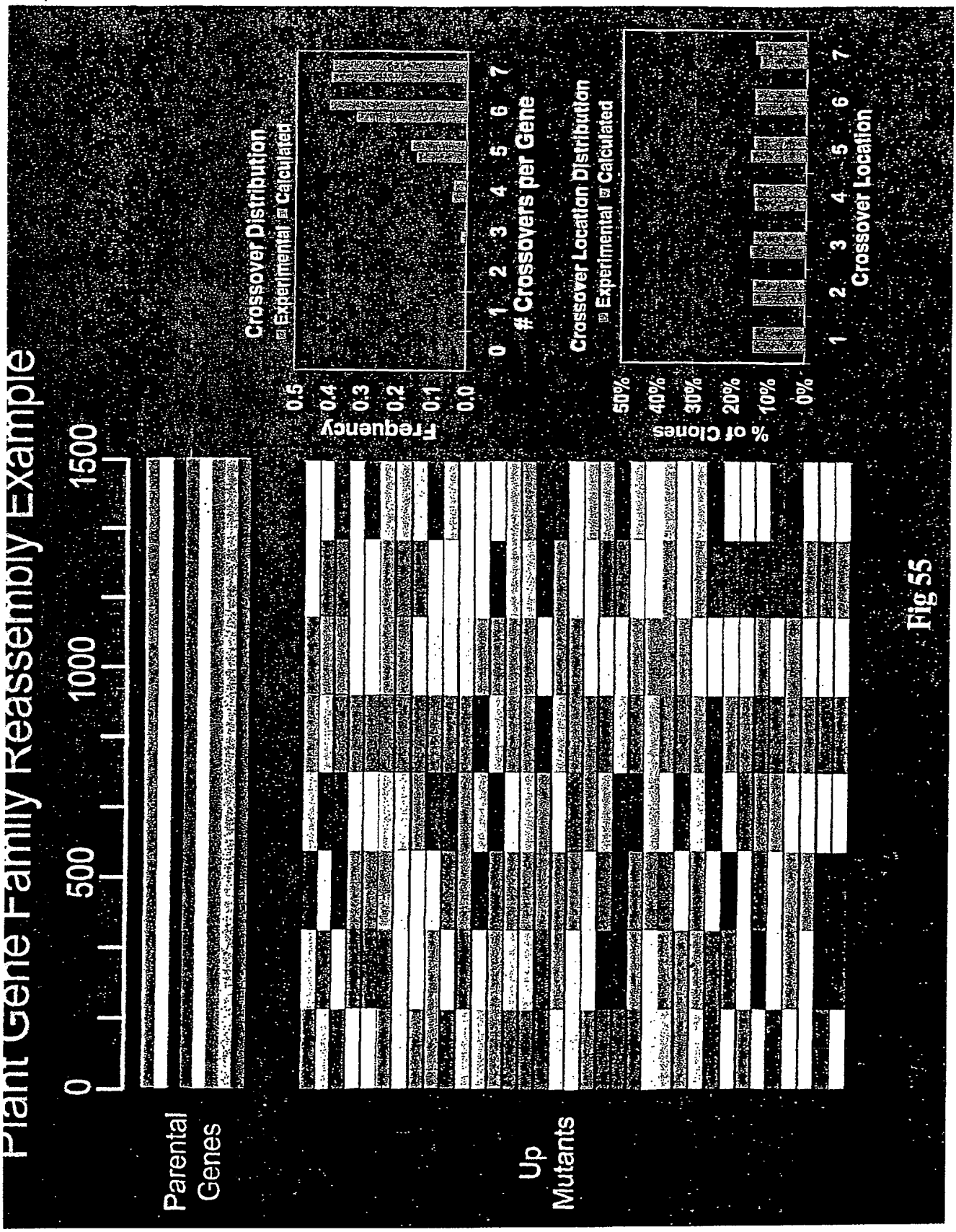

Plant Gene Family Reassembly Example
0
500
1000
1500
Parental Genes
Up Mutants
Crossover Distribution
Experimental
Calculated
Frequency
0.5
0.4
0.3
0.2
0.1
0.0
0 1 2 3 4 5 6 7
Crossovers per Gene
Crossover Location Distribution
Experimental
Calculated
% of Clones
50%
40%
30%
20%
10%
0%
1 2 3 4 5 6 7
Crossover Location

Fig 55

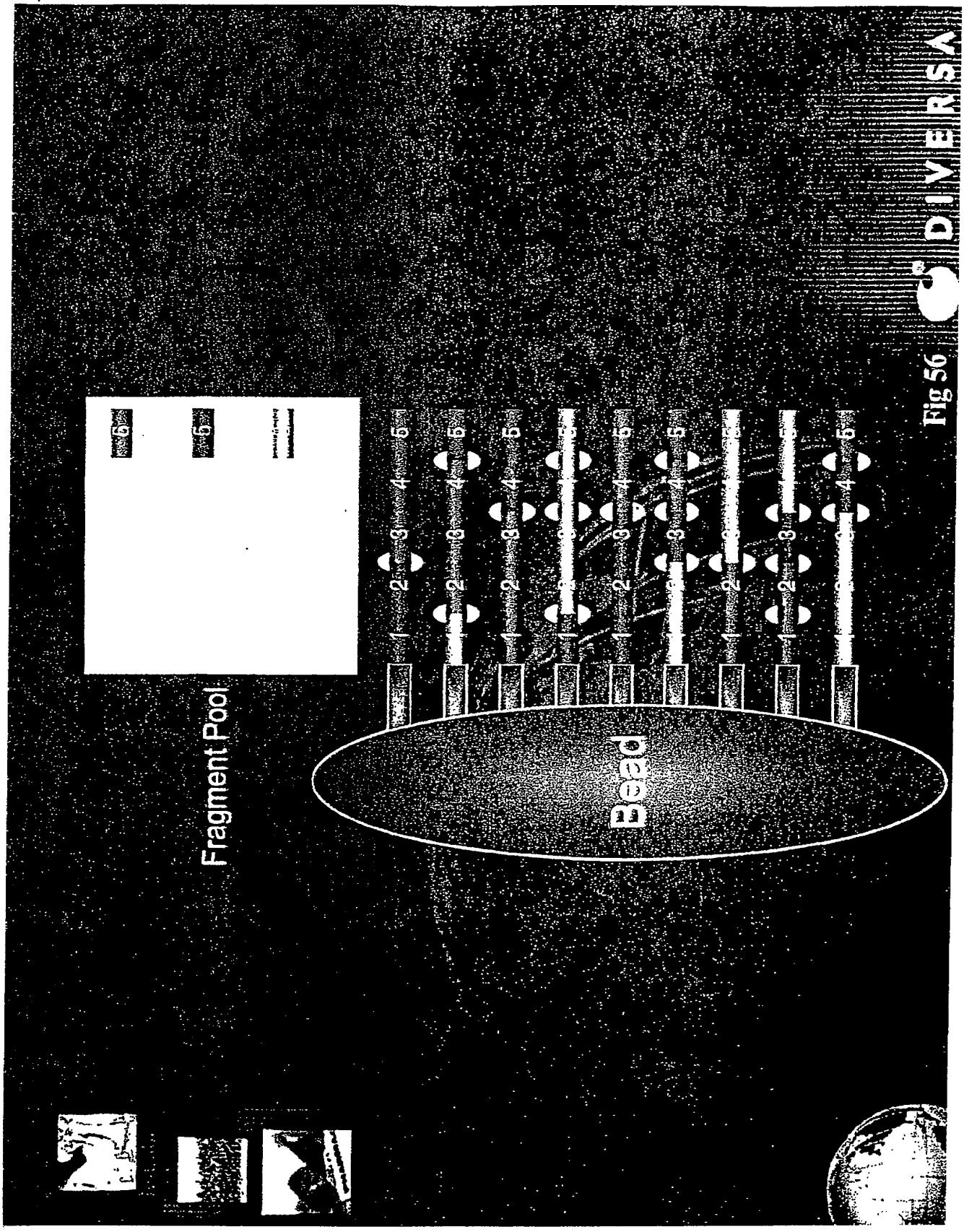


Fig 56

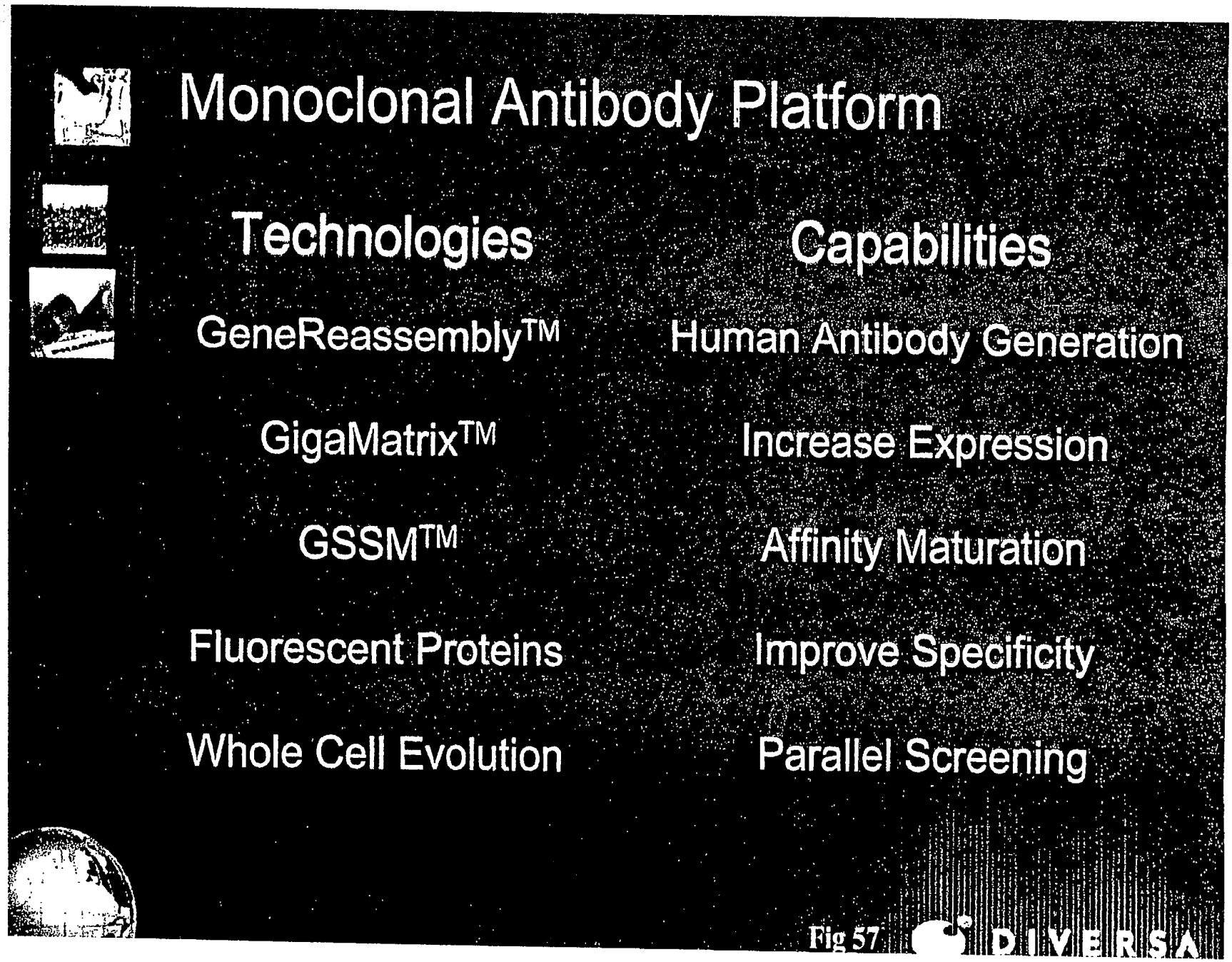

Monoclonal Antibody Platform
Technologies
GeneReassembly™
GigaMatrix™
GSSM™
Fluorescent Proteins
Whole Cell Evolution
Capabilities
Human Antibody Generation
Increase Expression
Affinity Maturation
Improve Specificity
Parallel Screening
DIVERSA

Fig 57

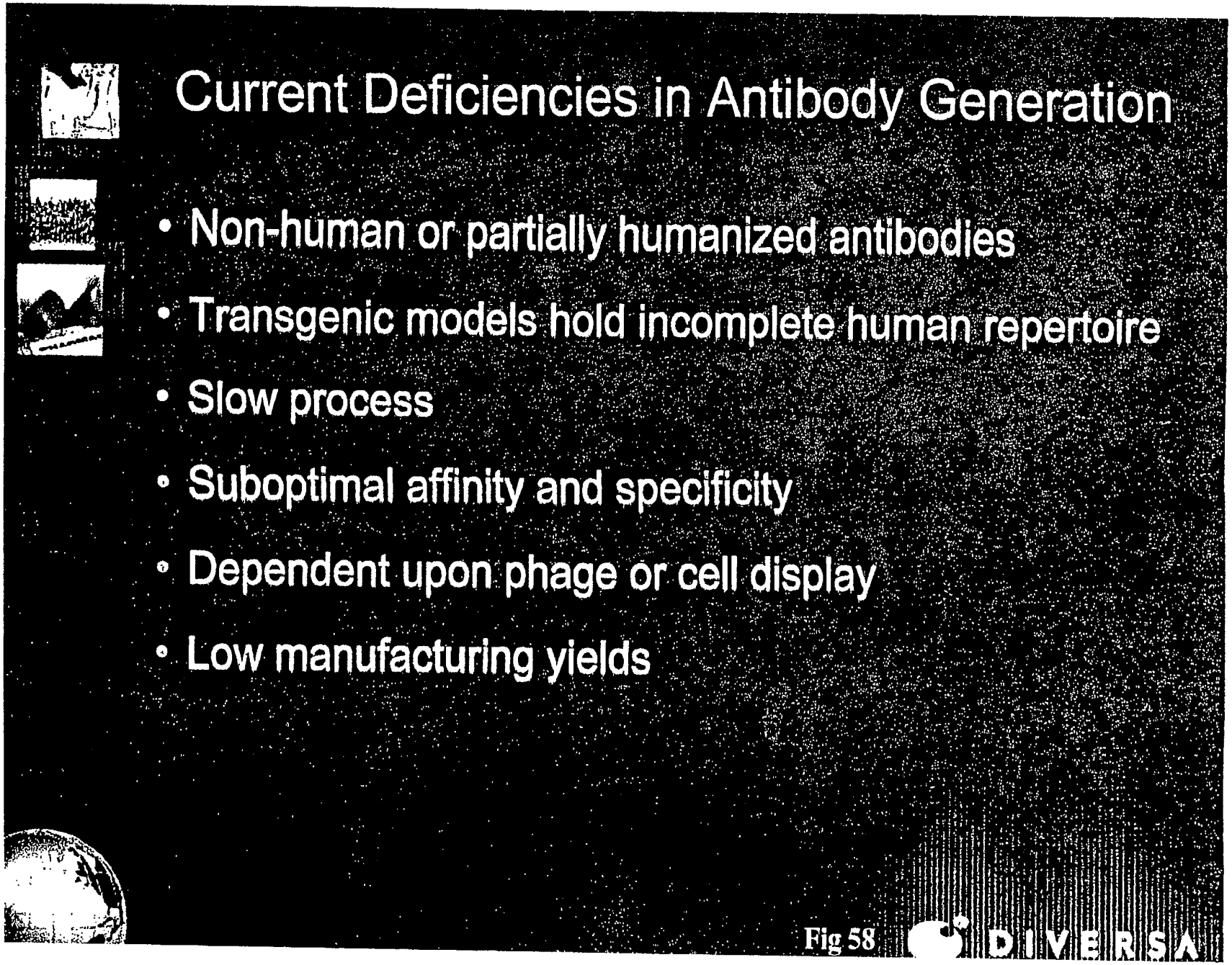

Current Deficiencies in Antibody Generation
• Non-human or partially humanized antibodies
• Transgenic models hold incomplete human repertoire
• Slow process
• Suboptimal affinity and specificity
• Dependent upon phage or cell display
• Low manufacturing yields
DIVERSA

Fig 58

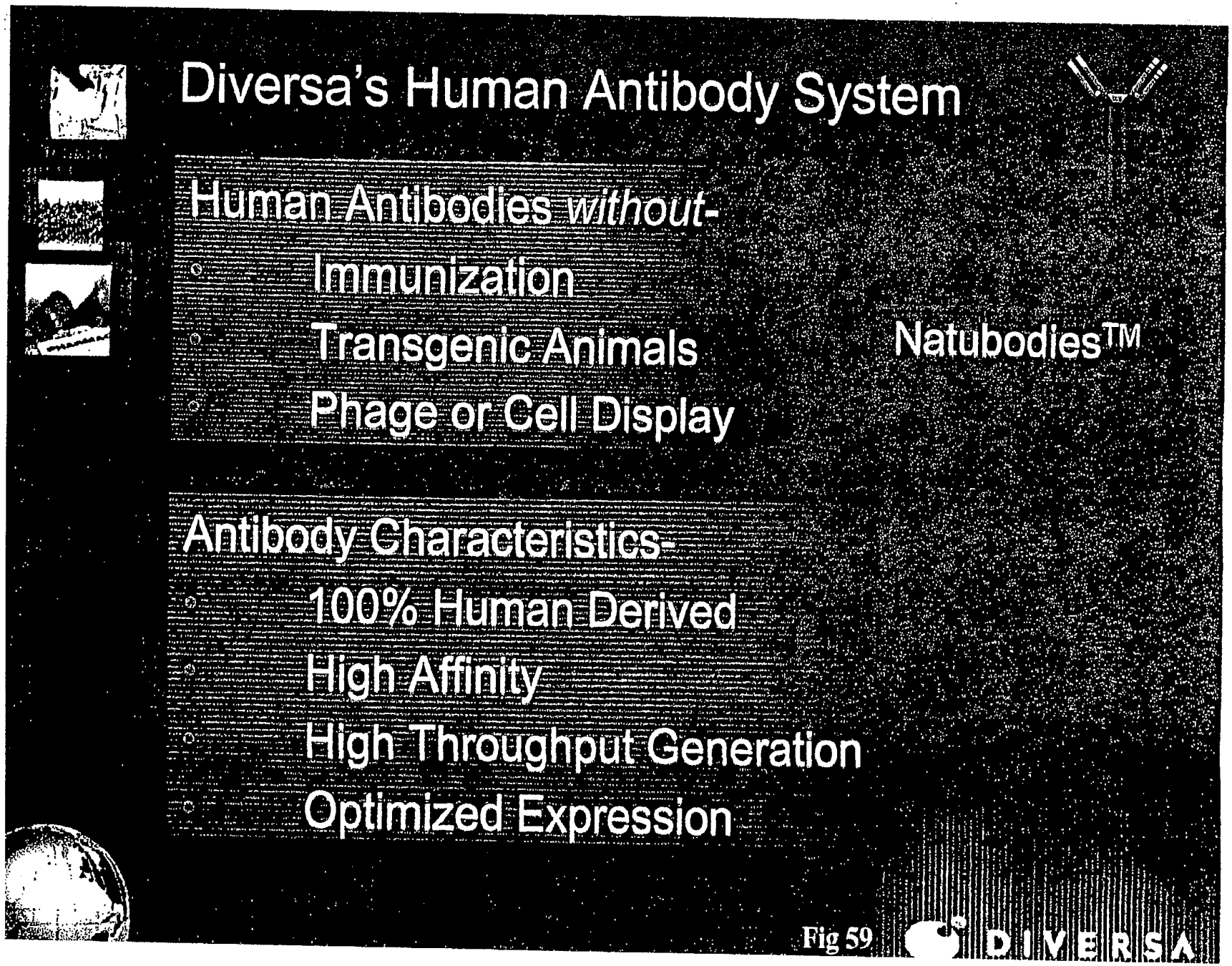

Diversa's Human Antibody System
Human Antibodies *without*-
Immunization
Transgenic Animals
Phage or Cell Display
Natubodies™
Antibody Characteristics-
100% Human Derived
High Affinity
High Throughput Generation
Optimized Expression
DIVERSA

Fig 59

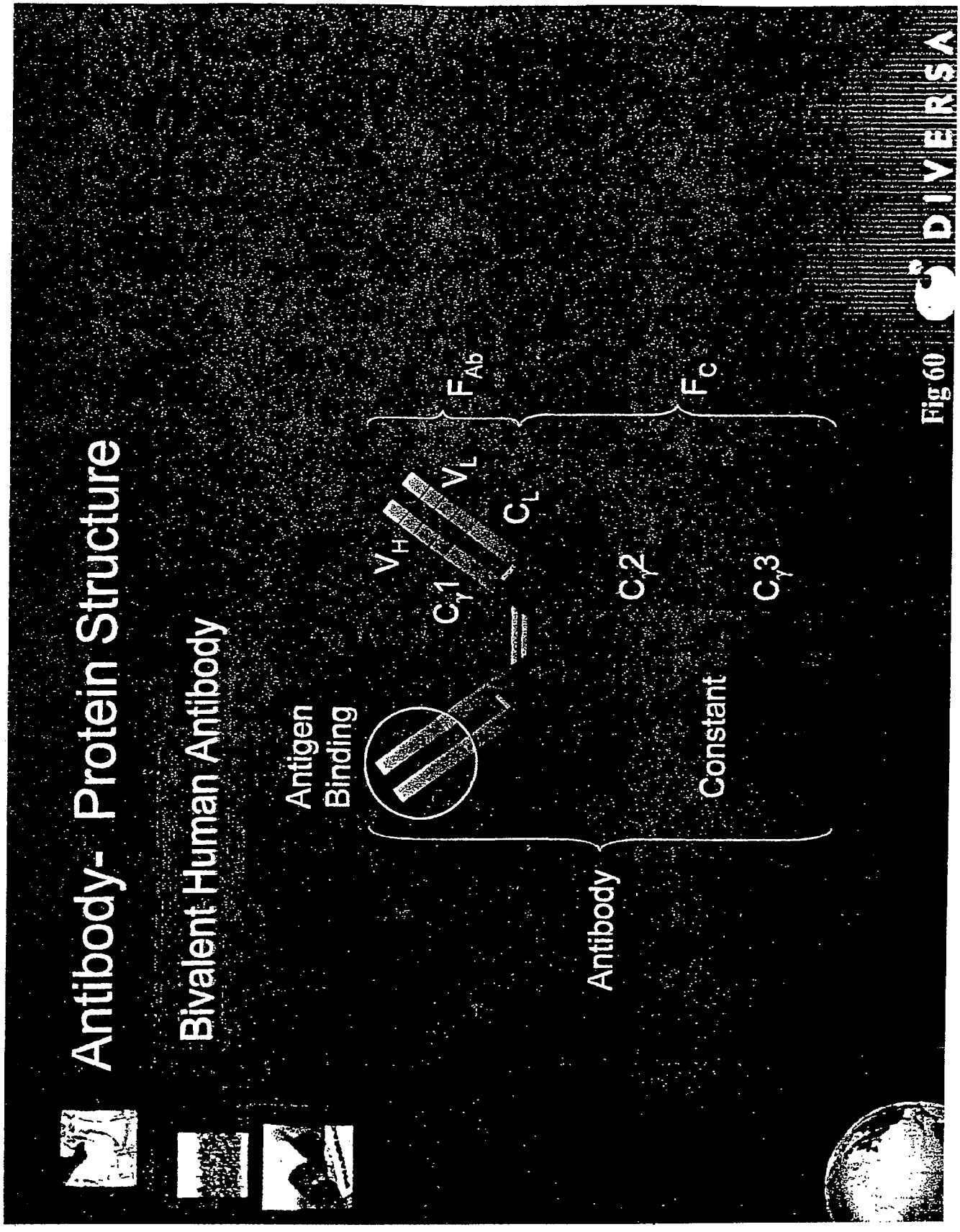

Antibody- Protein Structure
Bivalent Human Antibody
Antigen Binding
Antibody
Constant
V_H
V_L
$C_\gamma 1$
C_L
$C_\gamma 2$
$C_\gamma 3$
F_{Ab}
F_C
DIVERSA

Fig 60

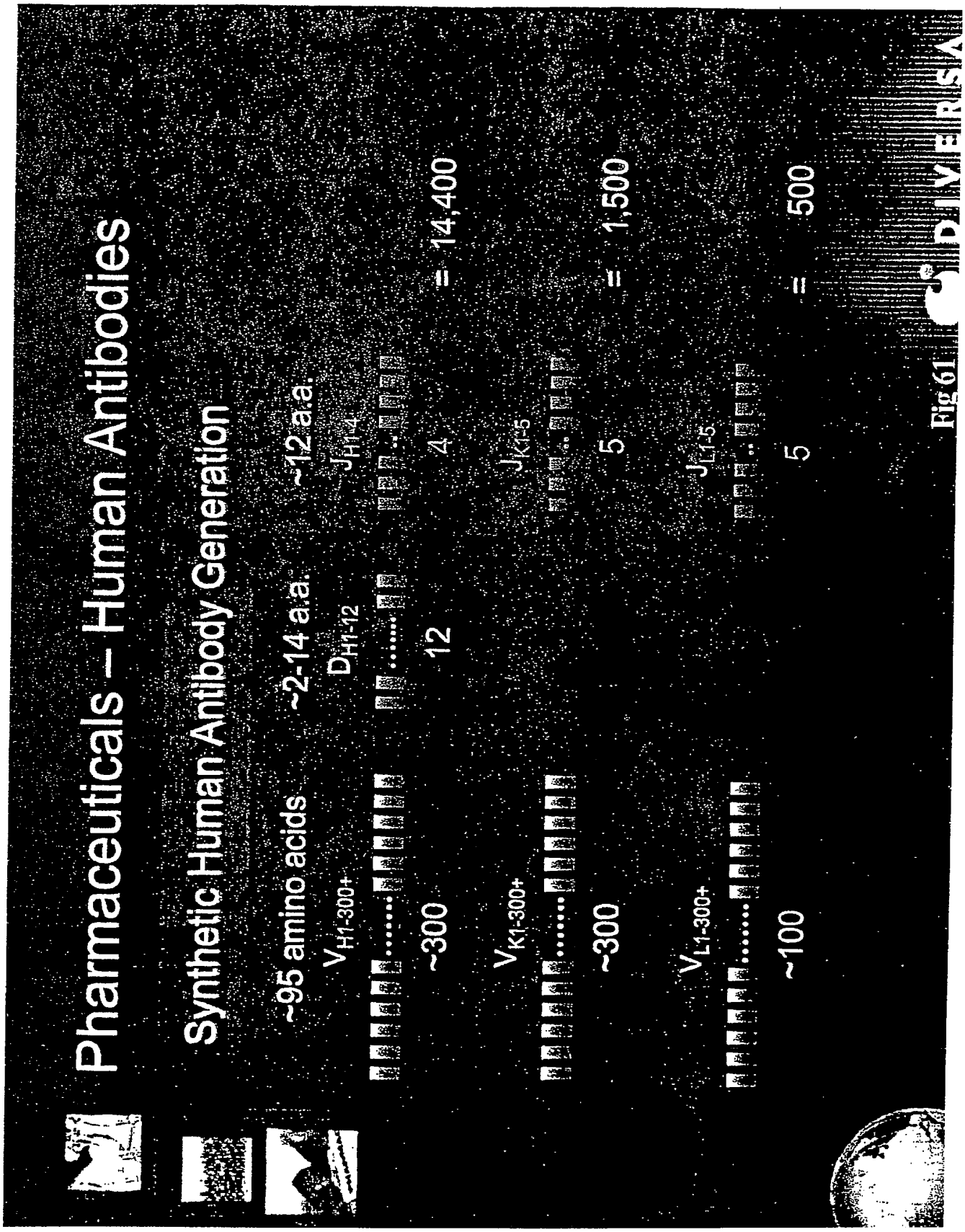

Pharmaceuticals – Human Antibodies
Synthetic Human Antibody Generation
~95 amino acids
~2-14 a.a.
~12 a.a.
$V_{H1-300+}$
D_{H1-12}
J_{H1-4}
~300
12
4
= 14,400
$V_{K1-300+}$
J_{K1-5}
~300
5
= 1,500
$V_{L1-300+}$
J_{L1-5}
~100
5
= 500

Fig 61

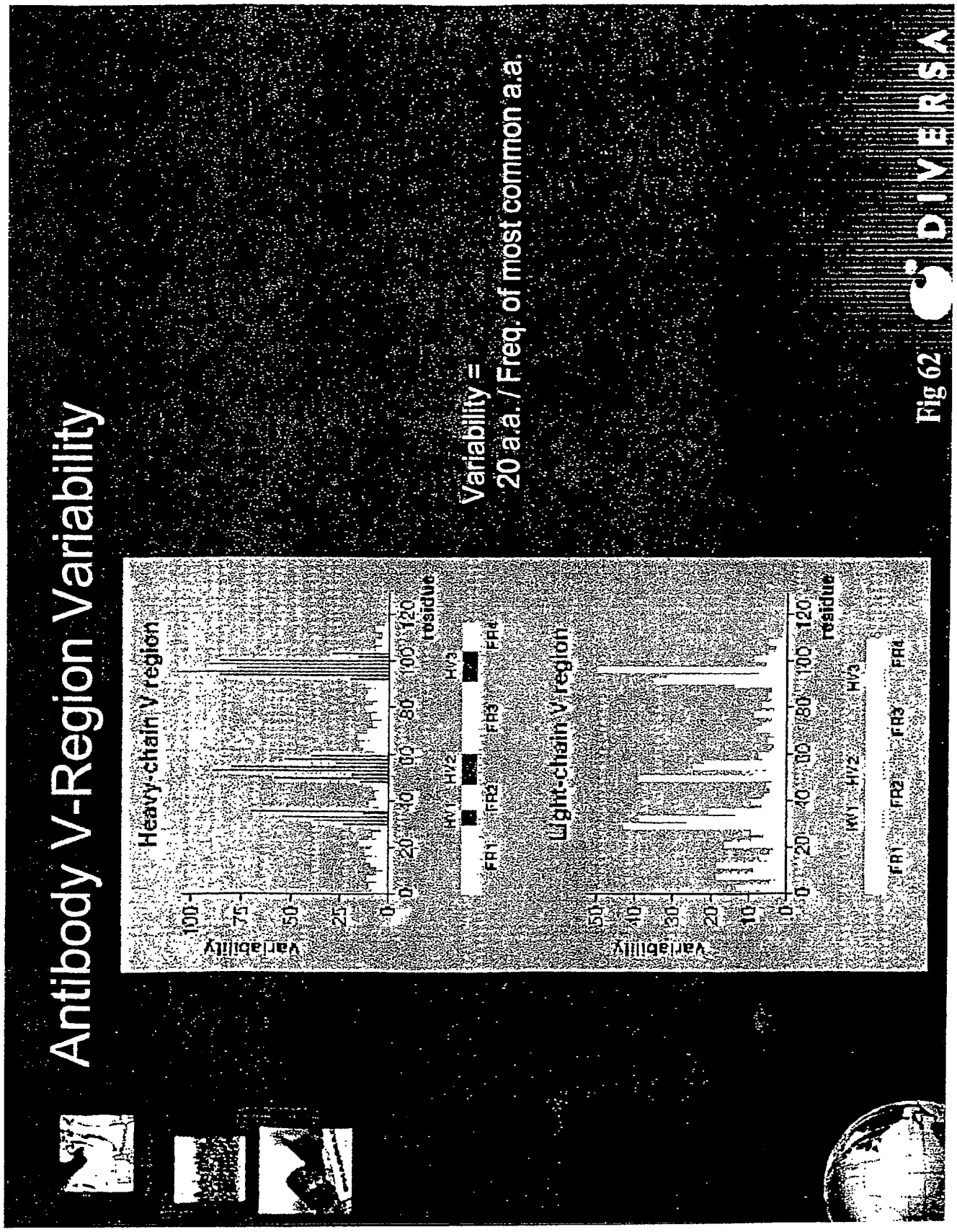

Antibody V-Region Variability
Heavy-chain V region
Variability
residue
FR1 H1 FR2 H2 FR3 H3 FR4
Light-chain V region
Variability
residue
FR1 H1 FR2 H2 FR3 H3 FR4
Variability =
20 a.a. / Freq. of most common a.a.
DIVERSA

Fig 62

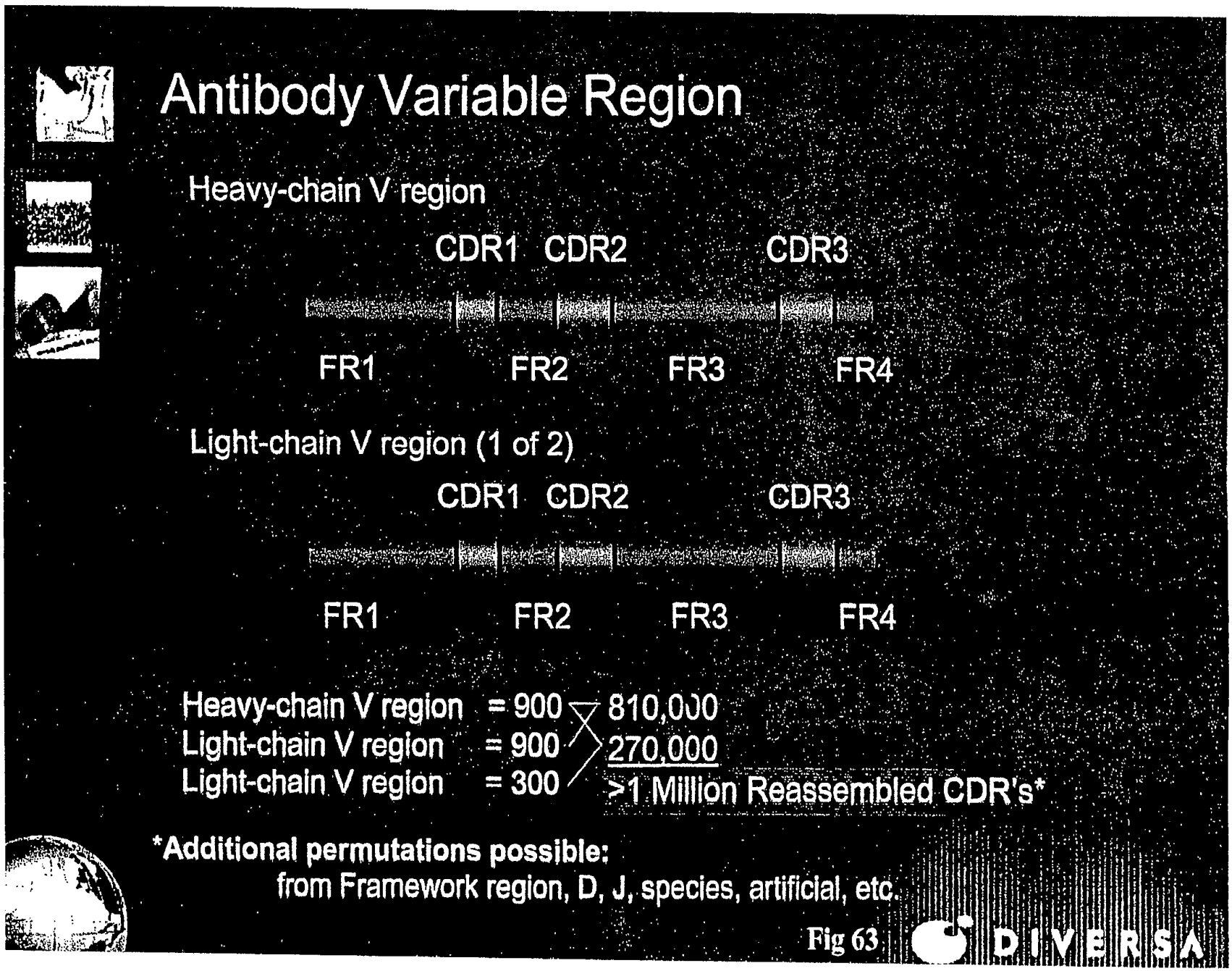
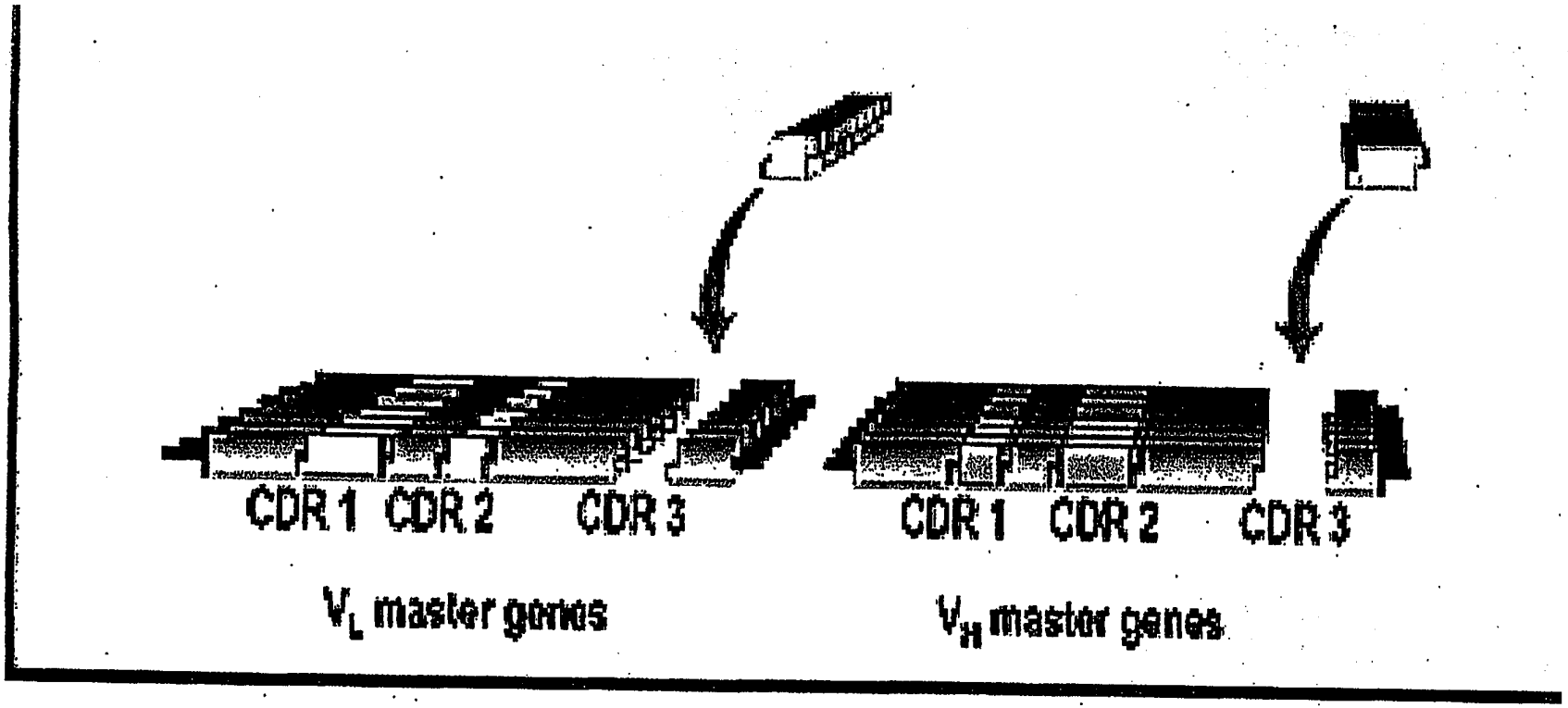

Antibody Variable Region
Heavy-chain V region
CDR1
CDR2
CDR3
FR1
FR2
FR3
FR4
Light-chain V region (1 of 2)
CDR1
CDR2
CDR3
FR1
FR2
FR3
FR4
Heavy-chain V region = 900
Light-chain V region = 900
Light-chain V region = 300
810,000
270,000
>1 Million Reassembled CDR's*
*Additional permutations possible:
from Framework region, D, J, species, artificial, etc.
DIVERSA

Fig 63



Diversity: 49 human antibody frameworks covering structural diversit

Affinity: Completely modular gene structure by de novo synthesis

Fig 64

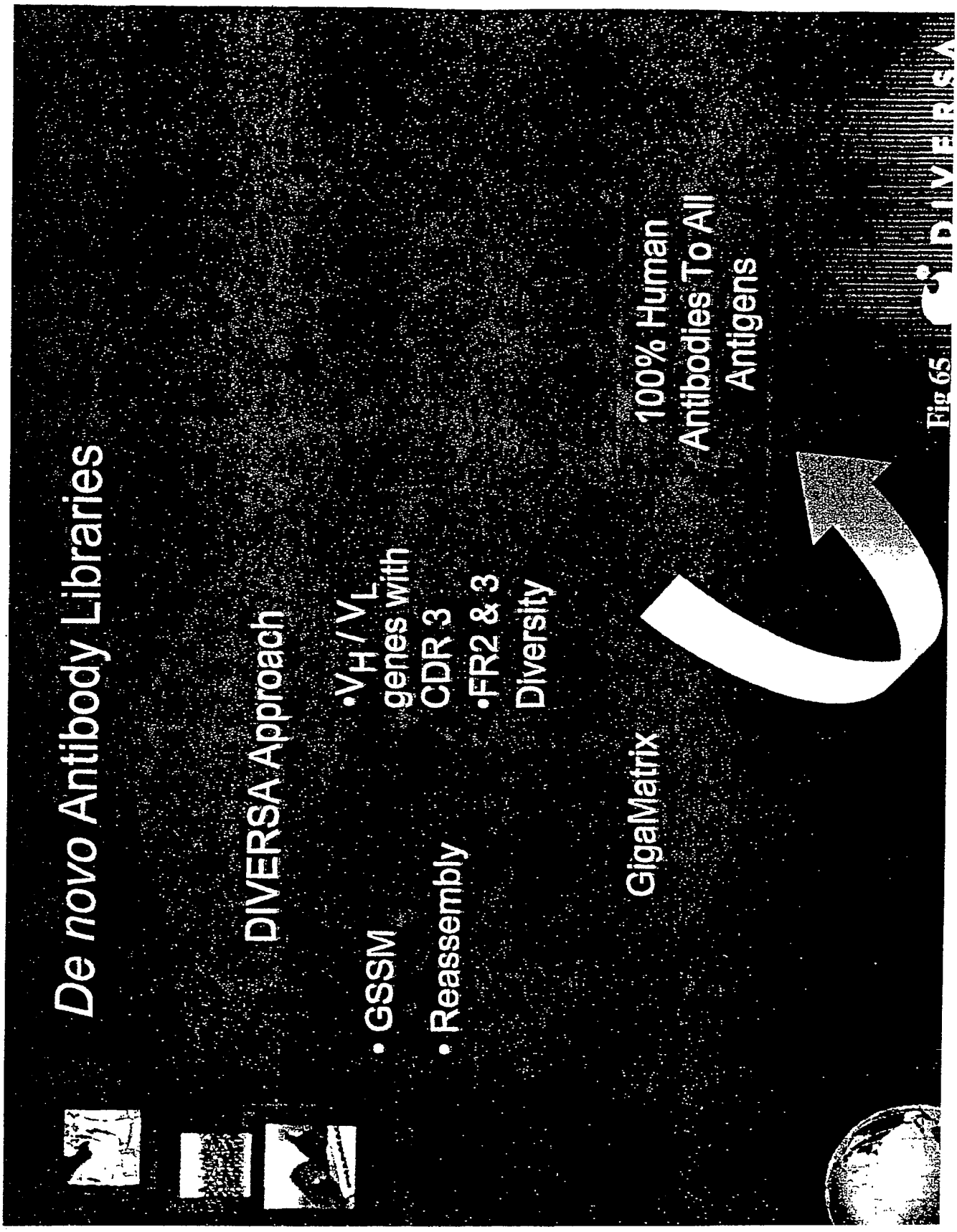

De novo Antibody Libraries
DIVERSA Approach
• GSSM
• Reassembly
• V_H / V_L genes with CDR 3 Diversity
• FR2 & 3 Diversity
GigaMatrix
100% Human Antibodies To All Antigens
DIVERSA

Fig 65

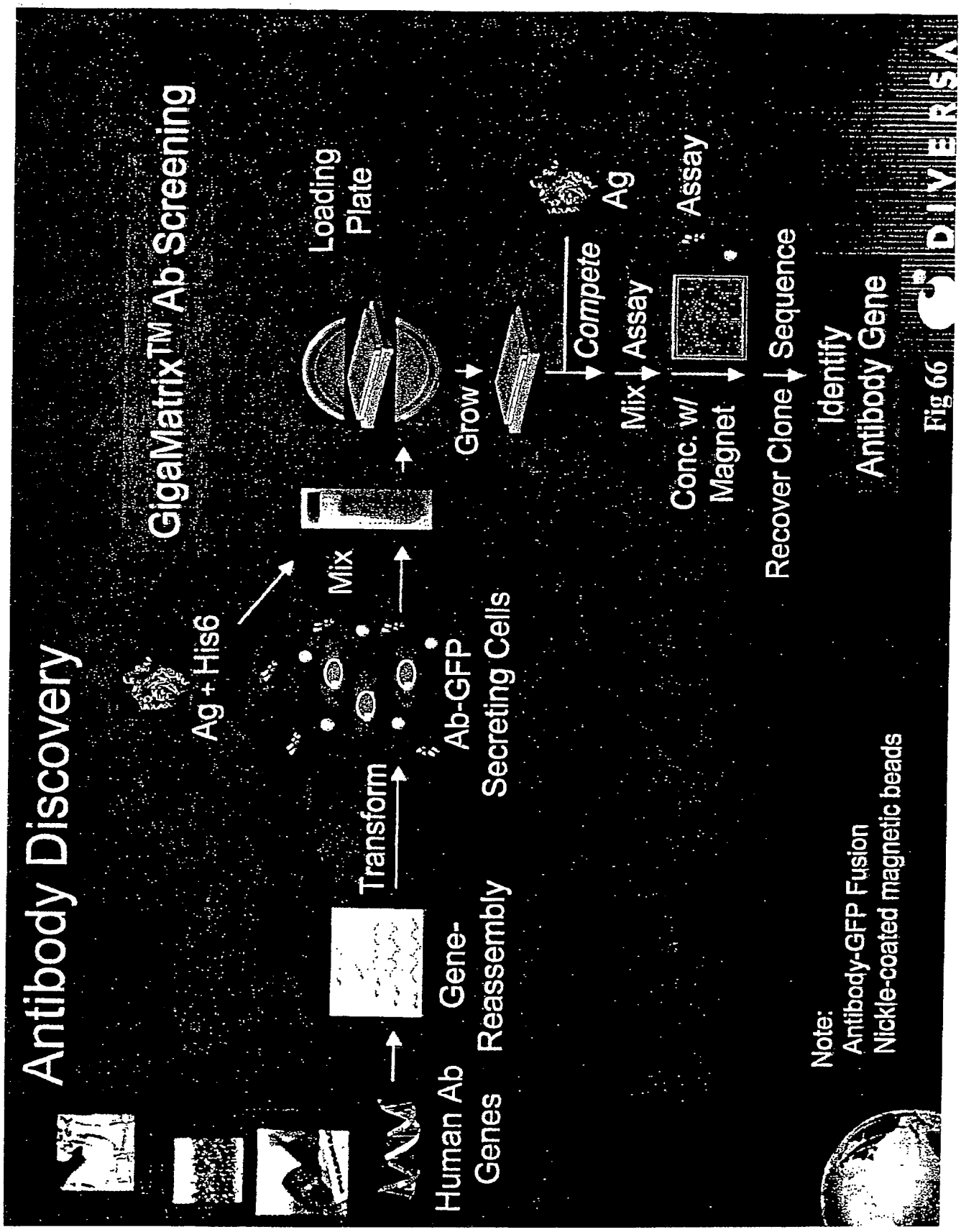

Antibody Discovery
GigaMatrix™ Ab Screening
Human Ab Genes
Gene-Reassembly
Transform
Ab-GFP Secreting Cells
Ag + His6
Mix
Loading Plate
Grow
Compete
Ag
Mix
Assay
Conc. w/ Magnet
Assay
Recover Clone
Sequence
Identify Antibody Gene
Note:
Antibody-GFP Fusion
Nickle-coated magnetic beads
DIVERSA

Fig 66

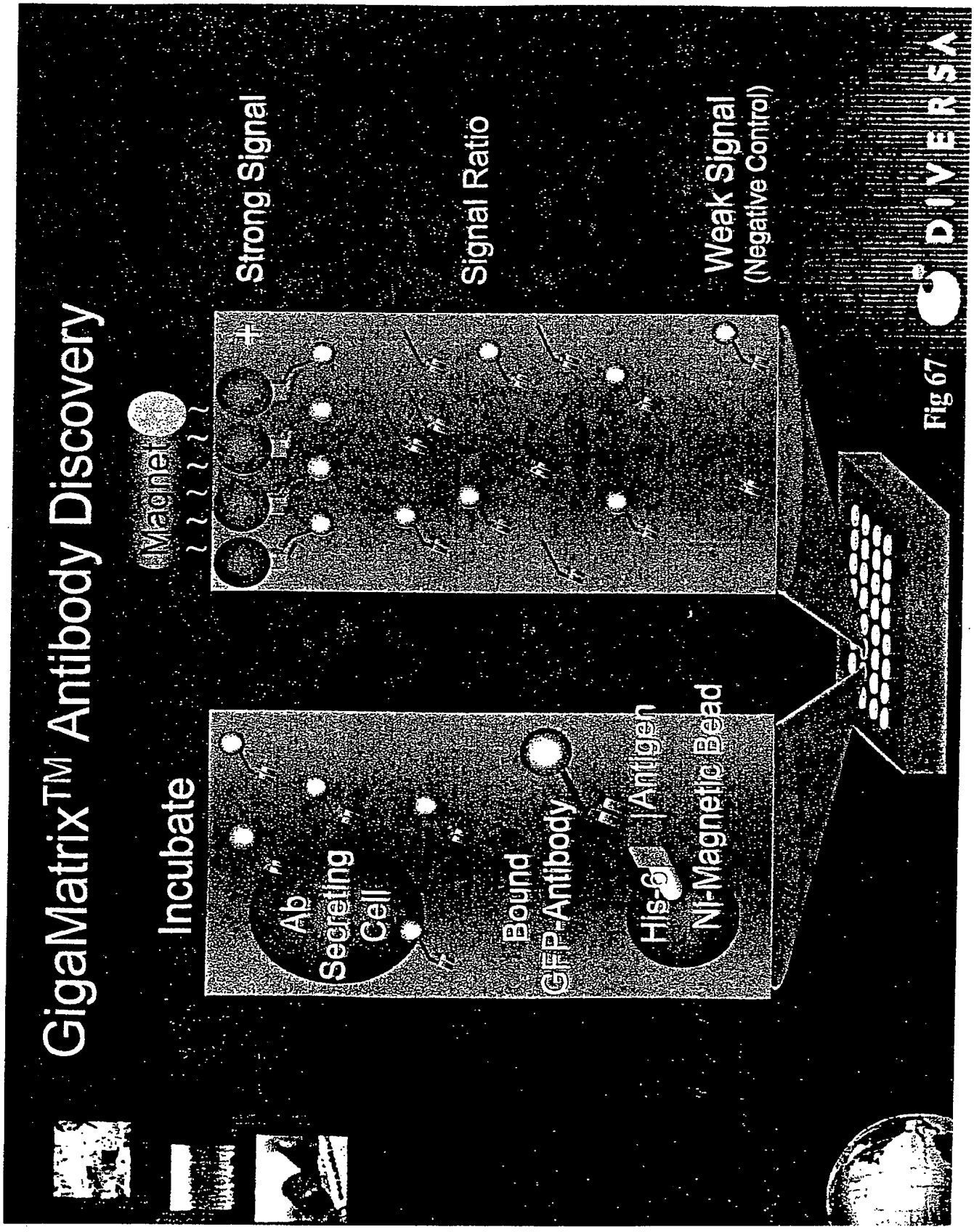

GigaMatrix™ Antibody Discovery
Incubate
Ab Secreting Cell
Bound GFP-Antibody
His-6
Antigen
Ni-Magnetic Bead
Magnet
Strong Signal
Signal Ratio
Weak Signal
(Negative Control)
DIVERSA

Fig 67

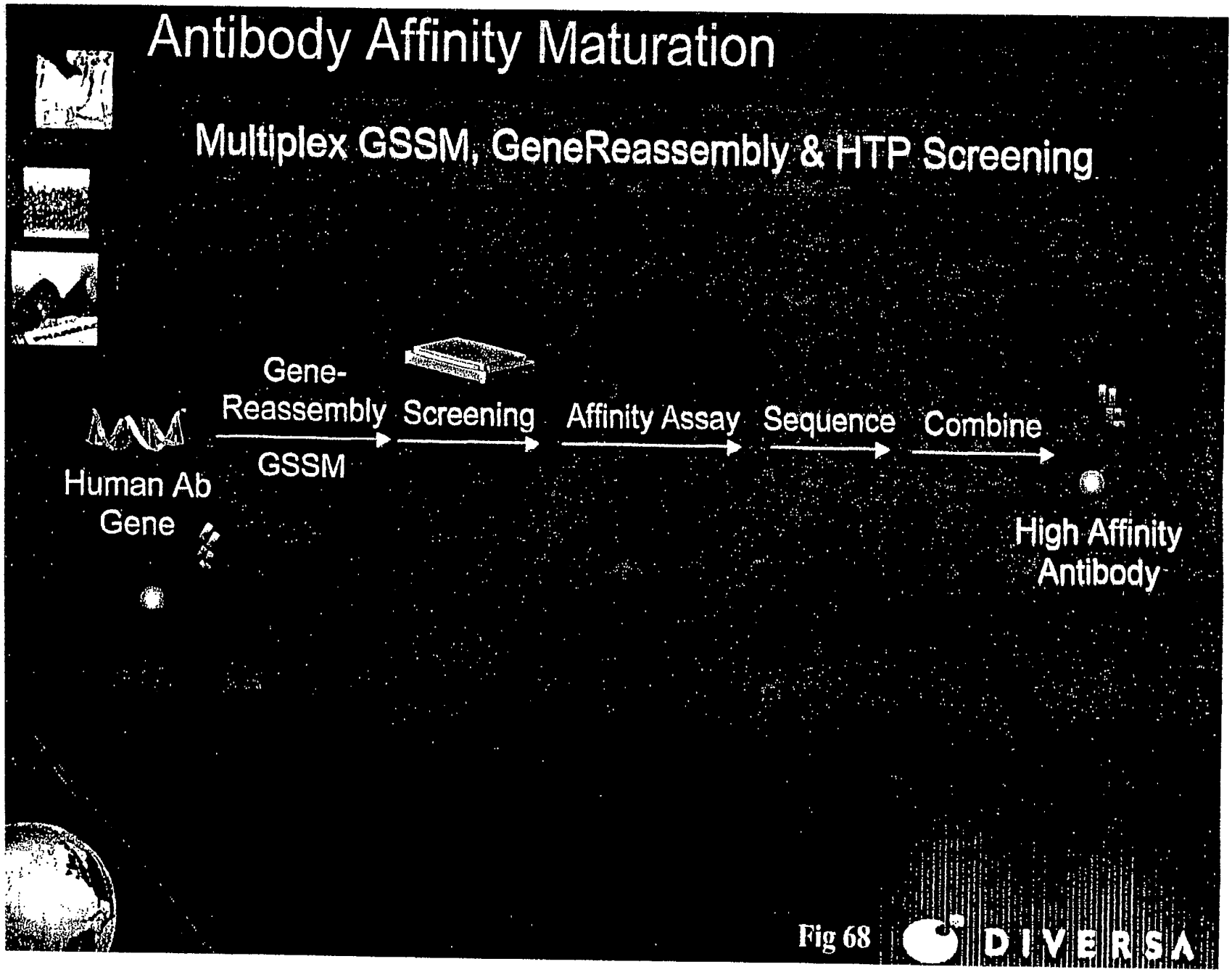

Antibody Affinity Maturation
Multiplex GSSM, GeneReassembly & HTP Screening
Gene-Reassembly
GSSM
Screening
Affinity Assay
Sequence
Combine
Human Ab Gene
High Affinity Antibody
DIVERSA

Fig 68

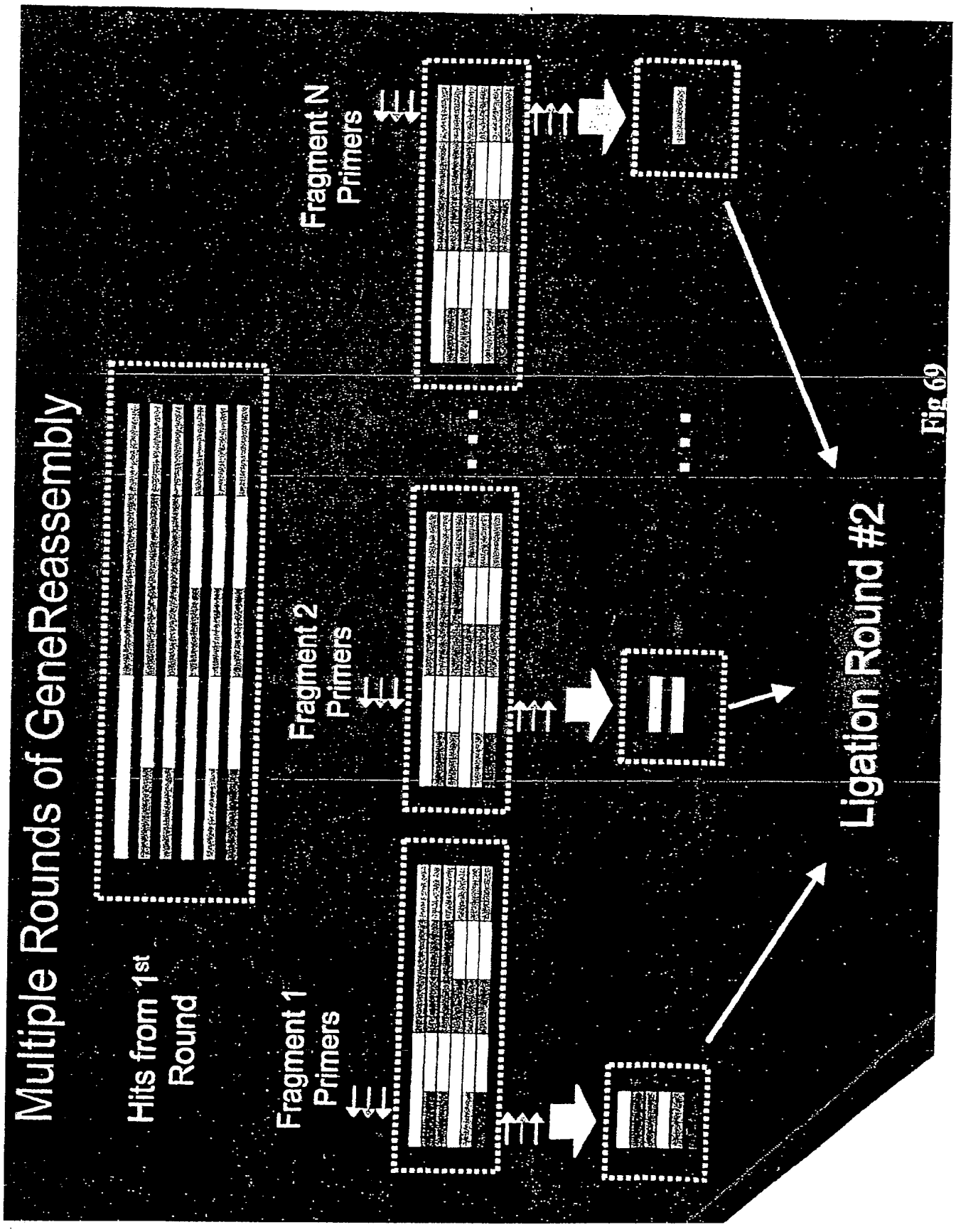

Multiple Rounds of GeneReassembly
Hits from 1st Round
Fragment 1 Primers
Fragment 2 Primers
Fragment N Primers
Ligation Round #2

Fig 69